EXHIBIT B

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The Tissue-Specific Expression of a Tobacco Phytochrome B Gene[1]

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We have isolated a genomic clone from *Nicotiana tabacum*, designated *Nt-PHYB-1*, encoding a type-II, "green tissue" phytochrome apoprotein. Recombinant genes, consisting of the 3319-bp promoter of the *Nt-PHYB-1* gene (including the entire 5′ untranslated sequence but not the ATG) or its deletion derivatives and the bacterial β-glucuronidase reporter gene, were constructed and transferred into tobacco. The expression patterns and levels of the endogenous *Nt-PHYB-1*, as well as those of the transgenes, were determined by RNase protection assays and by β-glucuronidase histochemical staining. We show that (a) the *PHYB-1* gene has three transcription start sites, (b) the abundance of the three *PHYB-1*-specific mRNAs is different, and that (c) it is not regulated by light. However, we do demonstrate that transcription of the endogenous *PHYB-1* gene and that of the recombinant genes exhibit a well-defined organ and tissue specificity. This tobacco *PHYB* gene is relatively highly expressed in leaf, stem, and different floral organs but not in root. Deletion analysis of the *Nt-PHYB-1* promoter indicates that a 382-bp region, located between −1472 and −1089, is required for high-level expression of this gene.

Throughout the life cycle of higher plants, photoreceptors, among them phytochrome, regulate a wide range of photomorphogenic responses, such as seed germination, flowering, and senescence. Recent studies have identified a number of distinct modes of photoregulation that are attributed to phytochrome, for example, the very-low-fluence response, low-fluence response, and high-irradiance response (Smith and Whitelam, 1990). These studies also showed that the expression of a number of plant genes is modulated by phytochrome through the photoreversible conversions between a Pr form and a Pfr form (Nagy et al., 1988a; Smith and Whitelam, 1990; Quail, 1991). Based on the heterogeneity of phytochrome-regulated genes and responses, it was postulated that there are multiple types of phytochrome and that these different types of phytochrome play different physiological roles during plant development. Indeed, recent biochemical and genetic evidence showed that the phytochrome apoproteins are encoded by a family of genes in *Arabidopsis thaliana* (Sharrock and Quail, 1989; Clack et al., 1995) and in other dicot and monocot plants (Quail, 1991). Three of the phytochrome genes in *A. thaliana* have been shown to be expressed differently, with *PHYA* encoding type-I, light-labile phytochrome A and the *PHYB* and *PHYC* encoding type-II, light-stable phytochromes B and C, respectively (Quail, 1991). Moreover, analysis of photomorphogenetic mutants lacking type A (phyA) (Nagatani et al., 1993; Parks and Quail, 1993; Whitelam et al., 1993) or type B (phyB) phytochromes (Reed et al., 1993) clearly established distinct, physiological roles for these different phytochrome molecules.

To gain further information about the physiological roles of these different phytochromes it will be necessary to determine their tissue/cell-specific expression patterns and levels throughout the life cycle of plants. Both in monocot and dicot plants, the expression of genes encoding type-A (light-labile) phytochrome is down-regulated by light, although to different extents (for reviews, see Quail, 1991; Furuya, 1993). In dicot plants, a single *PHYA* gene produces multiple transcripts that are differently regulated by light (Sato, 1988; Tomizawa et al., 1989; Adam et al., 1994, 1996; Dehesh et al., 1994). Localization and tissue-specific expression of type-A phytochrome has been studied immunocytologically in oat seedlings (for review, see Pratt, 1994) and, in greater detail, in transgenic seedlings and plants by GUS histochemical methods (Komeda et al., 1991; Adam et al., 1994; Somers and Quail, 1995a, 1995b). These latter studies established that expression of the dicot pea, tobacco (*Nicotiana tabacum*), and Arabidopsis *PHYA* genes is down-regulated by light and exhibits characteristic developmental and organ- and tissue-specific patterns.

In contrast, relatively little is known about the expression patterns of the *PHYB* genes in different plants during development. It was found that in potato, pea, and Arabidopsis the expression of these genes is not regulated by light (for review, see Furuya, 1993), and that the transcription of a *PHYB*-like gene in potato has multiple transcription start sites (Heyer and Gatz, 1992). In addition, the organ-specific distribution of the phyB protein was studied by immunocytology in monocots (Wang et al., 1993a, 1993b; for review, see Pratt, 1994). Very re-

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Abbreviations: D, darkness; *I*, intensity; L, light; MS, Murashige and Skoog.

cently, Somers and Quail (1995a, 1995b) characterized the temporal and spatial expression pattern of the *PHYB-GUS* transgene in transgenic *Arabidopsis thaliana* plants and seedlings. These latter authors reported that most cells appear to express the *PHYB-GUS* transgene at some level at all stages examined, with the highest apparent activity in vascular tissue and root tips. Apart from these studies, however, neither the transcription initiation nor the distribution of the *PHYB* mRNA or that of the phyB protein has been characterized in other plant species.

We are interested in obtaining detailed information on the spatial and temporal distribution of the tobacco *PHYB* mRNA during development. Therefore, we monitored the expression level and pattern of the endogenous tobacco *PHYB-1* gene (Kern et al., 1993) and those of the *PHYB-1-GUS* transgenes in transgenic plants from germination to flowering. We found that the transcription of this *PHYB* gene produces three transcripts in all tissues examined, and exhibits a well-defined tissue/cell-specific pattern. Furthermore, we show that an approximately 400-bp upstream region of the *Nt-PHYB-1* gene is required for high-level expression.

MATERIALS AND METHODS

Plant Material, Light Treatments

Nicotiana tabacum SRI plants were grown on sterile MS medium, supplemented with 3.0% Suc, or in soil in the greenhouse. Selected transgenic tobacco plants were maintained on sterile MS medium and supplemented with 3.0% Suc and with 100 mg/L kanamycin or grown in soil in the greenhouse. Unless otherwise indicated, all plants were grown under light/dark cycles (16 h of L/8 h of D; I = 20 W/m^2). Seedling material used in different experiments was always grown, after surface sterilization, under sterile conditions on MS medium supplemented with 3% Suc and with 100 mg/L kanamycin under 16-h-L/8-h-D cycles as described above. Alternatively, etiolated seedlings were irradiated with monochromatic red or far-red light as described previously by Adam et al. (1994). Light sources used in different experiments were as follows. For red light: L_{max}, 658 nm; I = 6.8 W/m^2; half-bandwidth, 15 nm. For far-red light: L_{max}, 730 nm; I = 3.5 W/m^2.

RNase Protection Experiments

Total RNA was isolated as described previously (Nagy et al., 1988b). RNase protection assays were performed as described (Sambrook et al., 1989), with the following modifications. Various fragments representing the 5′ untranslated leader region of the *PHYB-1* gene, namely a 317-bp *Hin*dIII-*Pst*I fragment, a 389-bp *Hin*dIII-*Bgl*I fragment, and a 490-bp *Bgl*I fragment, were cloned into pKS plasmid. These plasmids were linearized by *Xho*I digestion and used as templates to generate [α-^{32}P]UTP-labeled antisense PHYB-1 RNAs. Alternatively, a 150-bp fragment of a tobacco gene encoding the 18S rRNA was amplified by PCR and cloned into a pKS plasmid. This plasmid was linearized by *Sal*I digestion and used as template to generate labeled antisense 18S rRNAs. Production of the labeled antisense PHYB-1 and 18S rRNAs, hybridization, and RNase digestion were performed as described by Adam et al. (1994).

The level of the *PHYB-1* mRNA is relatively low, and to detect the various *PHYB-1* gene-specific transcipts we had to use 100 μg of total RNA in the RNase protection assays. The high amount of nonspecific RNA resulted, occasionally, in the incomplete digestion of the labeled probes and in the appearance of multiple weak bands (for example, see Fig. 2B, lane 3). We have employed different probes and multiple RNA samples, and repeated these RNase protection assays at least three times. We found that the appearance of these weak bands is not reproducible and, therefore, we concluded that they do not represent specific *PHYB-1* transcripts.

Recombinant DNA Techniques, Construction of the *PHYB-GUS-NOS* Chimeric Genes, and Plant Transformation

The promoter region of the *PHYB-1* gene was isolated and sequenced from genomic clones identified by screening a Charon 35 tobacco genomic library with a 300-bp DNA fragment that corresponds to the N-terminal region of the phyB protein. A 3319-bp fragment, containing the putative promoter region of the *PHYB-1* gene, was chosen for further studies. We constructed chimeric genes, by translational fusion, containing the *PHYB-1* promoter region or its 5′ deletion derivatives (but not the start codon ATG) fused to the *GUS-NOS* reporter gene (isolated from the pBI101.1 plasmid; Jefferson et al., 1987). The resulting chimeric genes were then cloned in the pMON 505 binary vector and transferred into tobacco by *Agrobacterium tumefaciens*-mediated transformation. Transgenic plants were selected as described by Fraley et al. (1985). All DNA manipulations, including sequencing and cloning, were performed according to Sambrook et al. (1989).

GUS S1-Nuclease Protection Experiments

GUS mRNA levels were determined by S_1-nuclease protection experiments. The labeled probe contained a 167-bp region of the *GUS* gene (downstream from the start codon ATG; for details, see Fejes et al., 1990) but no sequence homology to the 5′ untranslated regions of the tobacco *PHYB-1* gene. Therefore, this probe was used only to measure *GUS* mRNA levels. It was not suitable to map transcription start sites of the *PHYB-1-GUS* transgenes. This probe is very AT rich at its 5′ end and it is therefore likely that the relative instability of the DNA-RNA hybrids at this region could result in slightly different S_1-nuclease digestion patterns, i.e. in the appearance of two or three protected fragments that differ by only 1 to 3 bp.

GUS Activity Assays, GUS Histochemical Staining

Preliminary characterization of the expression level of the different transgenes was performed by GUS enzyme activity assays using 4-methyl-umbelliferyl-β-D-glucuronide as substrate. Histochemical localization of the GUS enzyme activity was carried out according to Adam et al. (1994) by using 5-bromo-4-chloro-3-indolyl-β-D-glucuronide as described by Jefferson (1988).

RESULTS

Transcription and Expression Pattern of the Endogenous *PHYB-1* Gene

Kern et al. (1993) recently reported the isolation of tobacco genomic clones encoding type-II phytochrome B apoprotein. To characterize the expression of this tobacco gene, designated *Nt-PHYB-1*, we determined the number, level, and distribution pattern of *PHYB-1* mRNA in seedlings and in fully developed, flowering plants. Initially, we monitored the steady-state *PHYB-1* mRNA levels by RNase protection assays in 6-d-old seedlings grown in 16-h-L/8-h-D cycles. We employed three different labeled probes corresponding to various regions of the 5′ untranslated sequence of the *PHYB-1* gene. Figure 1 shows that transcription of the *PHYB-1* gene produces three different transcripts. The abundance of these mRNAs is not equal. The major transcript (transcription initiation starts at −124) represents about 65% of the total *PHYB-1* mRNA. The contribution of the two minor transcripts to the *PHYB-1* mRNA level (transcription initiation sites are at −70 and at −210) is about 25 and 10%. Alternatively, we determined the steady-state *PHYB-1* mRNA levels in 6-d-old seedlings grown in constant dark or in constant dark but treated with short pulses of red, red/far-red, and far-red light, and returned to dark. Figure 2A shows that the levels of the different *PHYB-1* transcripts are not affected by these red/far-red-light treatments. This observation indicates that under these conditions, in contrast to the *PHYA* genes (Adam et al., 1994), the expression of the *PHYB-1* gene is not autoregulated by the photoreceptor phytochrome.

Expression levels of the *PHYB-1* gene were also measured in different organs of fully developed, flowering tobacco plants and in young, developing seedlings grown under 16-h-L/8-h-D cycles. Figure 2B indicates that the levels of the *PHYB-1*-specific mRNAs are highest in stem tissue. They are about 2-fold lower in leaves and about 4-fold lower in seedlings and in undissected flowers. Figure 2B also shows that the ratio of the three *PHYB-1*-specific transcripts (major transcript, 65%; the two minor transcripts, 25 and 10%, respectively), independent of the expression level, is identical in all organs examined. The expression level of the *PHYB-1* gene was also determined in each floral organ and in root. Figure 3B shows that it was highest in gynoecium and in petal (it was about as high in these tissues as in stem), and it was about 2-fold lower in androecium and sepals. Furthermore, Figure 3B shows that it was present at a very low level in root tissue. We again found that the ratio of the three *PHYB-1* mRNAs was similar in all tissues, except in root. In root tissue, accumulation of the two minor *PHYB-1*-specific mRNAs was below detection level (data not shown).

Expression Pattern of the *PHYB-1-GUS* Transgene in Transgenic Tobacco Plants

We defined the expression pattern of the *PHYB-1* gene at the tissue/cell-specific level. To this end, we constructed a *PHYB-1-GUS-NOS* chimeric gene by translational fusion. This construct contained the putative promoter of the *PHYB-1* gene (a 3319-bp fragment spanning the entire upstream region, including the full-length 5′ untranslated

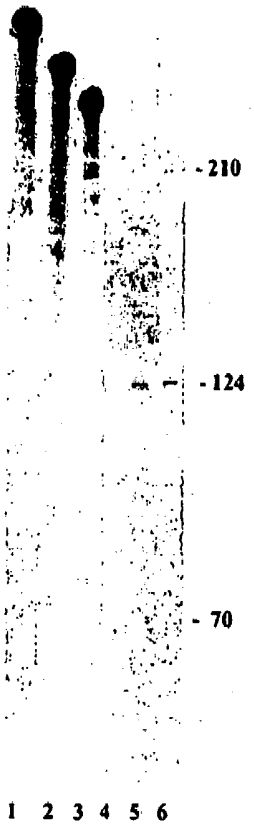

Figure 1. Transcription of the *PHYB-1* gene produces three distinct mRNA species. *PHYB-1*-specific mRNA levels were determined by RNase protection assays. The labeled probes (lanes 1–3) represent various regions of the 5′ untranslated region of the *PHYB-1* gene: from −879 to −389 (lane 1); from −389 to +1 (lane 2); and from −317 to +1 (lane 3). Probe 1 did not yield any protected fragment (lane 4). The protected 210-, 124-, and 70-nucleotide RNA fragments obtained by using probe 2 (lane 5) and probe 3 (lane 6) are indicated. Lanes 4 to 6 contain 100 μg of total RNA. Data from RNase protection assays were quantified by PhosphorImager (Molecular Dynamics, Sunnyvale, CA) using IMAGE, QUANT software, version 3.3 (Molecular Dynamics).

leader sequences from +1, but not the ATG) and the *GUS-NOS* reporter gene (Fig. 3A). Twenty-eight transgenic tobacco plants were regenerated and selfed, and the expression of each *PHYB-1-GUS* transgene was determined by GUS enzyme activity assays on F_1 progeny resistant to kanamycin. Table I shows that the majority of the selected plants, 21 out of 28, expressed this transgene, with the expression level varying by about 10-fold among the individual plants. For each regenerated transgenic plant, a further characterization of the expression pattern of the *PHYB-1-GUS* transgene was performed by S_1-nuclease protection experiments. We found (Fig. 3B) that the *GUS* mRNA levels differed characteristically in the various organs of all transgenic plants analyzed. Furthermore, Figure 3B also illustrates that organ-specific distribution of *GUS* mRNA parallels that of the *PHYB-1* mRNA.

Additionally, we monitored the *GUS* and the *PHYB-1* mRNA levels in light-grown or in dark-grown transgenic

seedlings for 6 d after sowing. Figure 4 shows that in seeds the *GUS* mRNA is below detection level. This figure also shows that levels of the *GUS* mRNA increase, in light-grown as well as in dark-grown seedlings, to reach similar maxima on d 4 after sowing. After 4 d, the steady-state *GUS* mRNA levels did not change significantly. In contrast, accumulation of the endogenous *PHYB-1* mRNA is low, but detectable, in seeds (Fig. 4). Furthermore, *PHYB-1* mRNA levels reach similar maxima, independent of growth conditions, 1 d after sowing. After the 1st d, the steady-state *PHYB-1* mRNA levels did not change and did not differ significantly in light- and dark-grown seedlings (Fig. 4).

The more detailed, tissue/cell-specific expression pattern of the *PHYB-1-GUS* transgene was then determined by GUS histochemical assays in transgenic seedlings or in flowering plants. We found no staining in seeds (Fig. 5a). Staining was restricted to the basal region of the cotyledons in 3-d-old dark-grown seedlings (Fig. 5b) and to the cotyledons in 7-d-old dark-grown seedlings (Fig. 5c). Figure 5 also shows that the root tips of the transgenic *PHYB-1-GUS* seedlings, independent of growth conditions and developmental stage, were never stained.

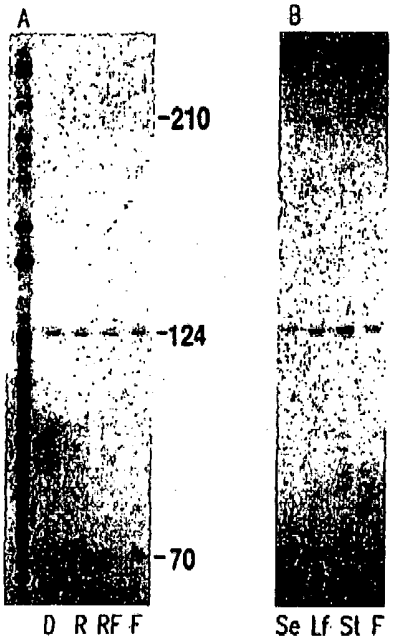

Figure 2. A, Transcription of the *PHYB-1* gene is not regulated by phytochrome. Seedlings were grown for 6 d in constant dark (D) illuminated with a 5-min pulse of red (R), 4 h of far-red (F), or 5 min of red followed by 4 h of far-red (RF) light, returned to dark, and harvested. *PHYB-1* mRNA levels were determined by RNase protection assays. The labeled probe (317 nucleotides) corresponds to the 5′ untranslated region of the *PHYB-1* gene from +1. The protected 210-, 124-, and 70-nucleotide RNA fragments are indicated. Each lane contains 100 μg of total RNA. The first lane shows the labeled molecular mass marker (pBR322 DNA digested with *Hpa*II). B, Abundance of the *PHYB-1* mRNA shows a characteristic organ specificity. Total RNA was isolated from seedlings or from different organs from flowering tobacco plants grown in 16-h-L/8-h-D cycles. Se, Seedling; Lf, leaf; St, stem; Fl, undissected flowers. *PHYB-1* mRNA levels were determined by RNase protection assays. Each lane contains 100 μg of total RNA. Data from these experiments were also quantified by PhosphorImager as described in the legend to Figure 1.

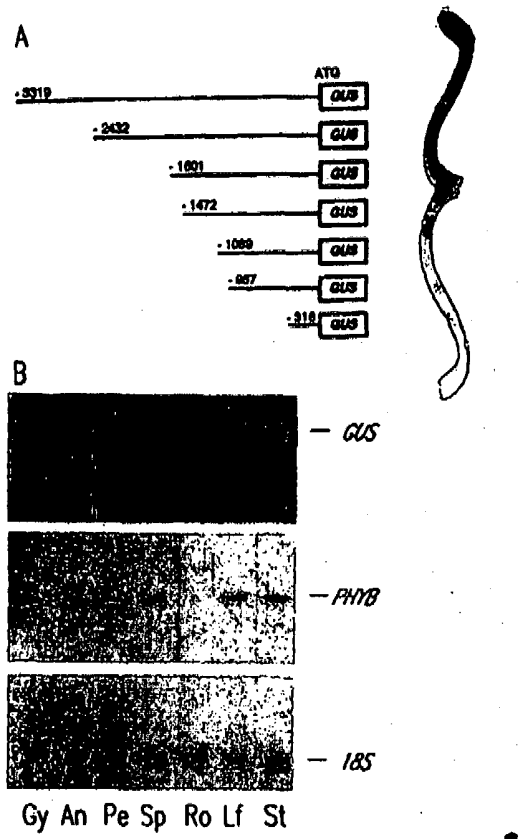

Figure 3. A, Structure of the chimeric *PHYB-1-GUS-NOS* constructs containing 5′ deletion derivatives of the *PHYB-1* promoter. Deletion mutants were produced by *Bal*31 digest as described by Sambrook et al. (1989). Numbering starts from the start codon ATG (+1) and indicates the 5′ end points of the promoter fragments. B, Expression pattern of the *PHYB-1-GUS-NOS* transgene is identical to that of the endogenous *PHYB-1* gene in transgenic tobacco plants. Gy, Gynoecium; An, androecium; Pe, petal; Sp, sepal; Ro, root; Lf, leaf; St, stem. Total RNA was isolated from different tissues of flowering transgenic plants grown in 16-h-L/8-h-D cycles. Aliquots of the same samples, containing 100 μg of total RNA, were used to determine the *GUS*, *PHYB-1*, and *18S rRNA* transcript levels by S_1-nuclease protection assays. The protected 107- (*GUS*), 124- (*PHYB-1*), and 150-nucleotide (*18S RNA*) fragments are indicated. Gels were exposed for 72 (*PHYB-1*, *GUS*) or 1 h (*18S rRNA*).

Expression of the *PHYB-1-GUS* transgene also showed a very characteristic pattern in mature, flowering transgenic plants. We detected relatively strong staining in petals, primarily around and in the veins, and in cells at the outer edge of the petal (Fig. 6, a and b). An even stronger color reaction was found in the stamen. Cells at the basal part of the filament and cells at the attachment site of filaments displayed particularly intense GUS staining (Fig. 6, c and d). It is interesting that we could also detect GUS staining in the stomium cells (Fig. 6d). GUS staining was also present in the style (Fig. 6e) and in the ovary (data not shown). Vegetative organs of the transgenic plants also exhibited heavily stained tissues. The spongy and palisade parenchyma cells of leaves showed an intense color reaction (Fig. 6f). Furthermore, we showed that

Table I. *The expression level and pattern of the PHYB-1-GUS-NOS and mutant PHYB-1-GUS-NOS chimeric genes in transgenic tobacco plants*

GUS activity: ++, 100–50; +, 45–10 pmol 4-methyl-umbelliferyl-β-D-glucuronide min^{-1} μg^{-1} protein; –, below detection level.

Construct *PHYB-1-GUS*[a]	No. of Plants	GUS Activity			Photo regulation	Tissue Specific
		++	+	–		
[−3319]	28	11	10	7	No[b]	Yes[c]
[−2432]	22	9	9	4	No[b]	Yes[c]
[−1601]	22	9	6	7	No[b]	Yes[c]
[−1472]	27	9	9	9	No[b]	Yes[c]
[−1089]	15	0	1	14	No[d]	No[d]
[−967]	14	0	0	14	No[d]	No[d]
[−316]	10	0	0	10	No[d]	No[d]

[a] All chimeric genes were constructed by translational fusion and contain, in addition to the promoter regions, the 5′ untranslated leader sequences (from +1) of the *PHYB-1* gene. Numerals in brackets indicate the 5′ end points of the *PHYB-1* promoter fragments fused to the *GUS-NOS* reporter gene cassette. [b] Determined by S_1-nuclease protection assay of RNA extracted from 6-d-old seedlings. [c] Monitored by GUS histochemical assay in flowering transgenic plants. [d] Not detectable by GUS S_1-nuclease protection or GUS histochemical assay.

incubation of transverse sections of petioles/leaf midribs (Fig. 6i) and stem (Fig. 6, g and h) resulted in complex GUS staining patterns. We found that specific phloem elements, most likely primary and secondary phloem cells, were heavily stained. These phloem elements showed particularly intense, "polarized" staining in the regions of stem close to branching sites (Fig. 6g). In contrast, Casparian strings, xylem elements of the vascular bundles, cortices, epidermal layers, and trichomes (as in the leaf) did not exhibit a significant GUS activity. GUS staining was absent from young roots and root tips in all 28 transgenic plants analyzed. We occasionally detected a very low level of GUS staining in the outermost layers of cells (rhizodermis) of the root hair zone (Fig. 6j).

Expression Level and Pattern of the *PHYB-1* 5′ Deletion Mutants

We showed that the expression pattern of the *PHYB-1-GUS-NOS* gene, containing a 3319-bp fragment of the tobacco *PHYB-1* gene, is very similar to that of the endogenous *PHYB-1* gene. We are interested in defining the *cis*-acting regulatory elements of the *PHYB-1* promoter required for the high-level and regulated expression of this tobacco gene. To this end, we constructed chimeric genes containing 5′ deletion derivatives of the 3319-bp *PHYB-1* promoter fused to the *GUS-NOS* reporter gene (Fig. 3A). The chimeric genes were introduced, again by *A. tumefaciens*-mediated transformation, into tobacco, and a large number of transgenic tobacco plants were generated (Table I). The expression levels and patterns of these mutants were then monitored by S_1-nuclease assays and by GUS histochemical staining.

Our data indicate that sequences located between −3319 and −1472 in the promoter region of the *PHYB-1* gene do not play an important role in the regulated expression of this gene. The deletion of this region did not affect either the level or the tissue specificity of the *PHYB-1-GUS-NOS* gene expression. The tissue-specific and light-insensitive expression patterns of these mutants were identical to that of the endogenous *PHYB-1* gene (data not shown). An additional deletion of the next 383 bp (from −1472 to −1089) resulted in a dramatic drop in the expression level of the *GUS* reporter gene. Out of 15 regenerated transgenic plants, we found detectable GUS enzyme activity in only 1 case. The very low expression level of this mutant made it impossible to characterize the expression features of this transgene by either S_1-nuclease protection or by GUS histochemical staining. Furthermore, deletion of *PHYB-1* promoter sequences completely abolished GUS enzyme activity (Fig. 3A; Table I).

DISCUSSION

We provide evidence that transcription of the tobacco *PHYB-1* gene occurs from three different initiation sites that are localized 70, 124, and 210 nucleotides upstream

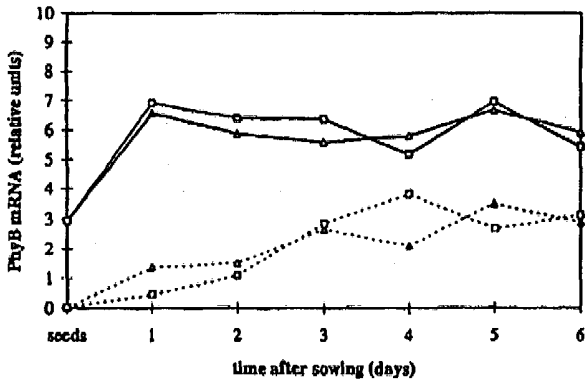

Figure 4. Transcription of the *PHYB-1* and that of the *PHYB-1-GUS* transgene is not regulated by light. Transgenic seedlings were sown and grown in constant light (Δ) or in constant darkness (■). Total RNA was extracted from seeds and from seedlings harvested daily during a 6-d period. *PHYB-1* (—) and *GUS* (- - -) mRNA levels were determined, in the same total RNA sample, by RNase and S_1-nuclease protection assays, respectively. *PHYB-1* mRNA levels represented by the 210-, 124-, and 70-nucleotide protected fragments and *GUS* mRNA levels represented by the 107-nucleotide protected fragments were quantified as described in the legends to Figure 2 (*PHYB-1*) and Figure 3 (*GUS*), and the calculated mRNA levels were plotted. The kinetics of the three *PHYB-1*-specific mRNA species accumulation was identical. This figure shows the accumulation pattern of the major *PHYB-1* transcript.

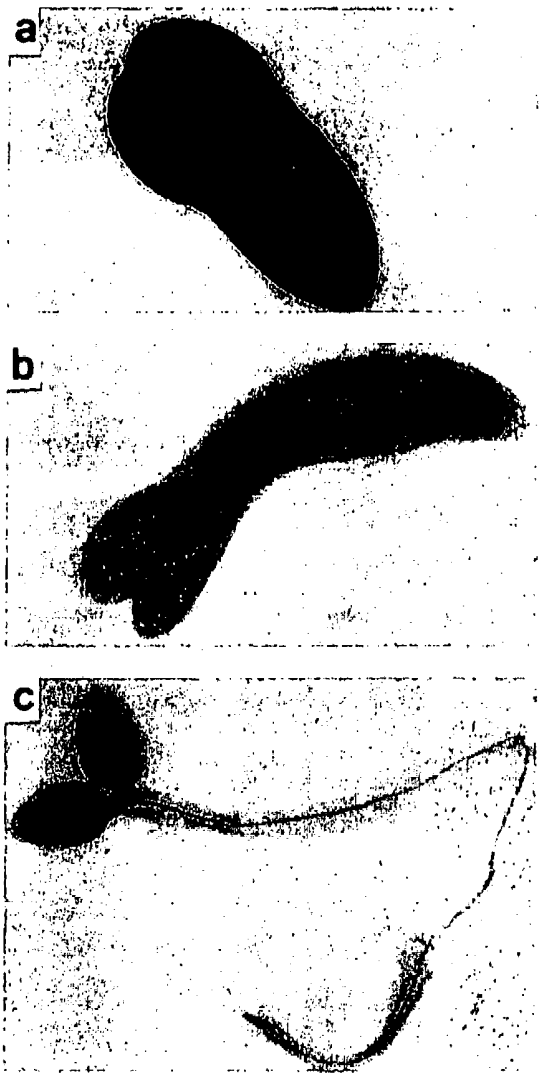

Figure 5. The expression pattern of the *PHYA-1-GUS* gene in developing transgenic tobacco seedlings. The chimeric genes contained the full-length (3319 bp) *PHYB-1* promoter fragment. GUS staining was performed on dry seeds (a) or on seedlings grown in constant dark for 96 h (b) or for 168 h (c).

from the ATG. Using primer extension we concluded that the region (approximately 1 kb) preceding the ATG start codon of the *PHYB-1* gene does not contain an intron(s). However, assuming there is no intron in the 5′ untranslated leader region of the *PHYB-1* gene, we found a TATA-box-like motif (TATAAA, 24 bp upstream of the putative start site) for only the most abundant *PHYB-1* transcript (initiated 124 nucleotides upstream of the ATG). No TATA-box-like motifs can be identified in the vicinity of the predicted start sites for the two minor *PHYB-1* gene-specific transcripts. Similar results were reported by Heyer and Gatz (1992) by monitoring the transcription initiation sites for a potato *PHYB* gene (which shows about 90% homology to the tobacco *PHYB-1* gene).

Results recently reported by McCormack et al. (1993) and Wester et al. (1994) suggest that (a) the abundance of phytochrome B is passively controlled by the number of transcription units (i.e by the *PHYB* mRNA level); (b) the activation of signaling systems regulating cell growth quantitatively depends on the concentration of phytochrome B Pfr molecules; and (c) small differences in *PHYB* gene expression level could initiate quite different responses in different cells. Furthermore, data reported by Wester et al. (1994) and by Somers and Quail (1995a, 1995b) suggest that a 2.1-kb promoter region of the *A. thaliana PHYB* gene contains all of the regulatory sequences required for proper spatial and temporal expression of phytochrome B. Also, these authors found that transcription of the *PHYB-GUS* transgene is down-regulated by light in transgenic *A. thaliana* seedlings.

We show that a 3319-bp promoter fragment of the tobacco *PHYB-1* gene is sufficient to express the *GUS* reporter gene in a manner similar to that of the endogenous *PHYB-1* gene, and a 387-bp sequence (located between −1472 and −1089) is required for high-level and regulated expression. In contrast to data reported by Somers and Quail (1995a, 1995b), however, we show that the expression of either the endogenous *PHYB-1* or the *PHYB-1-GUS* transgene is not down-regulated by light. In addition, we found that these genes are not expressed at detectable levels in root tissue. Of even more interest, we found that the kinetics of the *GUS* and *PHYB-1* mRNA accumulation are different in germinating young seedlings. This finding suggests that mRNA stability could also be a factor in regulating expression of the *PHYB-1* gene during early stages of plant development.

It is tempting, although quite speculative at present, to establish a direct relationship between the tissue/cell-specific expression pattern of the tobacco *PHYB-1* gene and the morphological changes described by Reed et al. (1993) in the mutant *A. thaliana*. It is conceivable that the localization of *PHYB-1* promoter activity in the specialized phloem cells (in stem, petiole, and leaf midrib) could indicate a role for phytochrome B in regulating the translocation of assimilates or signal molecules, which could affect cell elongation and flowering. The relatively high expression of the tobacco *PHYB-1* and the Arabidopsis *PHYB* genes in different flower organs could indicate the involvement of phytochrome in controlling early floral development. Similar to the *PHYA* genes, expression of the *PHYB* genes in chloroplast-containing cells most likely reflects the functions of this photoreceptor in the development of photosynthetic competence and in chloroplast morphogenesis (Bowler et al., 1994a, 1994b).

The very low level or the lack of detectable *PHYB-1* mRNA and promoter activity in tobacco root tissue is in contrast with the postulated role of phytochrome B in root elongation in *A. thaliana* (Reed et al., 1993).

Figure 6. (On facing page.) Tissue-specific expression pattern of the *PHYB-1-GUS-NOS* in flowering transgenic tobacco plants grown in 16-h-L/8-h-D cycles. The transgene contained the full-length (3319 bp) *PHYB-1* promoter fragment. a and b, Petal; c and d, pollen sac and stamen, respectively; e, style; f, leaf; g and h, stem; i, midrib of developed leaf; j, root. Magnifications are 17.2× (a–e, g, and j) and 43× (f and h).

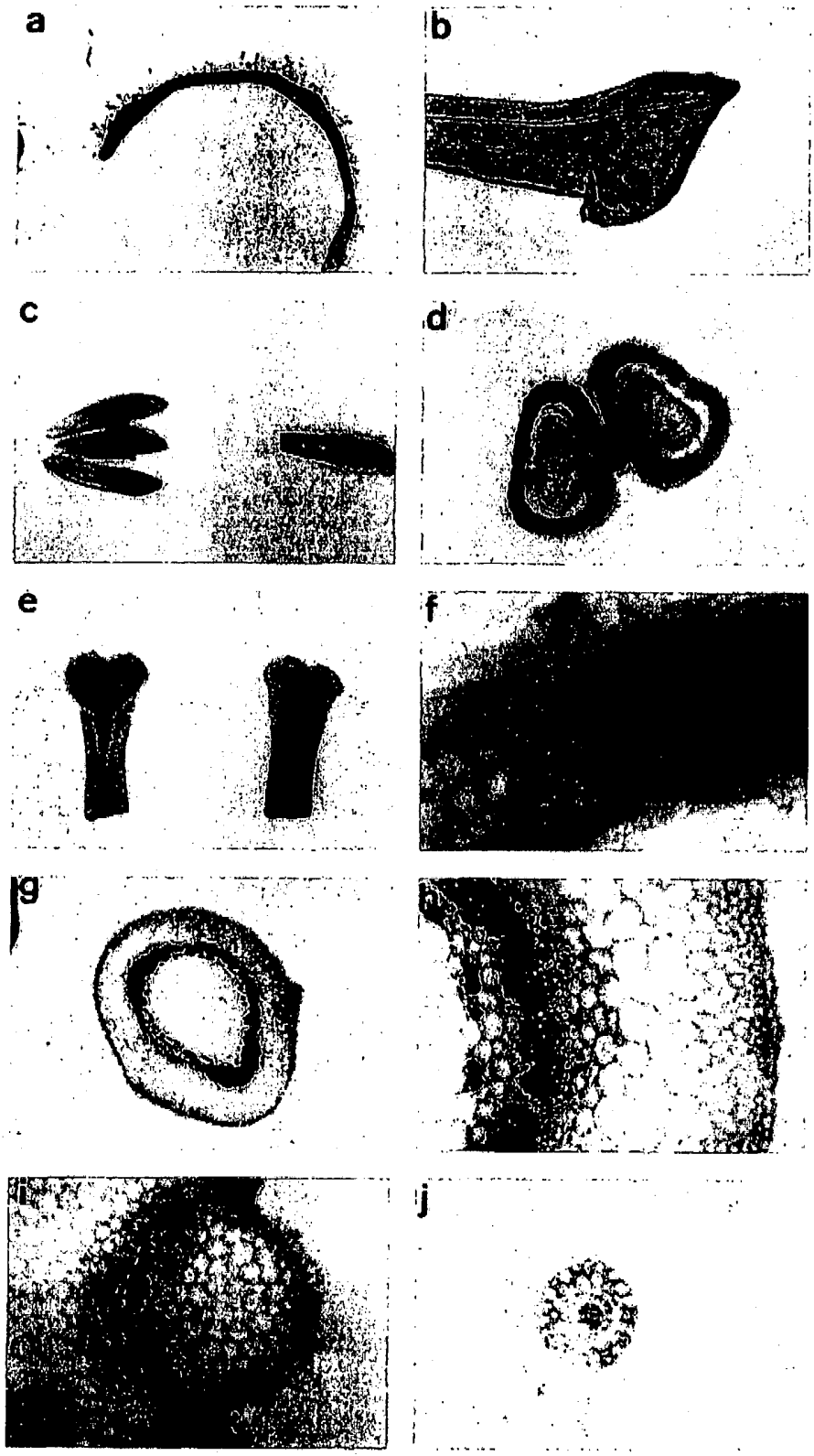

Figure 6. (Legend appears on facing page.)

The differential expression patterns of the *At-PHYB* and the *Nt-PHYB-1* genes, however, can be explained by assuming that (a) tobacco, like tomato (Pratt et al., 1995), may contain a more complex phytochrome gene family (i.e. several *PHYB*-like genes) and (b) the expression patterns and/or biological function of these different tobacco *PHYB* genes may differ slightly from each other and from that of the *PHYB* gene in *A. thaliana*. However, more work has to be done to determine the exact number, expression patterns, and functions of genes coding for phytochrome B-like proteins in tobacco and potato.

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